ELECTRONIC SUPPLEMENTARY MATERIAL

Assessment of stability of drug biomarkers in municipal wastewater as a factor influencing the estimation of drug consumption using sewage epidemiology

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Table S1 List of drug biomarkers and surrogate standards used in this study and the applied LC-MS-MS parameters

DTR	Abbreviation	Ionisation polarity	Retention time / min	Precursor ion (m/z)	Product ion 1 (m/z)	CE(1)/ V	Product ion 2 (m/z)	CE(2)/ V
Analyte:								
Morphine	MOR	ESI +	5.4	286	165	34	201	21
6-Acetyl morphine	6-AM	ESI +	11.6	328	165	34	211	24
Morphine-3-β-D-glucuronide	MG	ESI+	3.1	462	286	27	201	38
Benzoylecgonine	BE	ESI +	17.1	290	168	16	77	51
Cocaine	COC	ESI +	18.7	304	182	16	82	27
Amphetamine	AMP	ESI +	6.9	136	91	17	119	9
MDMA[a1]	MDMA	ESI +	10.8	194	163	11	135	21
Methamphetamine	MAMP	ESI +	8.6	150	91	17	65	38
THC-COOH[a2]	THC-COOH	ESI -	19.1	343	245	30	191	35
THC-OH [a3]	THC-OH	ESI -	17.9	329	311	24	173	33
Methadone	MTHD	ESI +	25.7	310	265	14	105	24
EDDP[a4]	EDDP	ESI +	24.6	278	234	27	249	20
Codeine	COD	ESI +	10.1	300	165	38	215	21
6-Acetyl codeine	6-AC	ESI+	18.8	342	165	42	225	23
Deuterated surrogates:								
Morphine–d_3	MOR-d_3	ESI +	5.4	289	165	37	201	24
6-Acetyl morphine-d_3	6-AM-d_3	ESI +	11.6	331	165	38	210	24
Morphine-3-β-D-Glucuronide-d_3	MG-d_3	ESI+	3.1	465	289	29	204	38
Benzoylecgonine-d_3	BE-d_3	ESI +	17.1	293	171	17	77	38
Cocaine-d_3	COC-d_3	ESI +	18.7	307	185	18	85	26
Amphetamine-d_3	AMP-d_3	ESI +	6.9	139	92	18	122	8
MDMA-d_5[b1]	MDMA-d_5	ESI +	10.8	199	165	12	135	21
Methamphetamine-d_5	MAMP-d_5	ESI +	8.6	155	92	18	91	17
THC-COOH-d_3[b2]	THC-COOH-d_3	ESI -	19.1	346	248	31	302	23
THC-OH- d_3[b3]	THC-OH-d_3	ESI -	17.9	332	314	23	173	33
Methadone-d_3	MTHD-d_3	ESI +	25.7	313	268	12	105	30
EDDP-d_3[b4]	EDDP-d_3	ESI +	24.6	281	237	28	249	20
Codeine-d_3	COD-d_3	ESI +	10.1	303	165	37	215	25
6-Acetyl codeine-d_3	6-AC-d_3	ESI+	18.8	345	225	22	165.03	43

CE = collision energy; ESI + electrospray ionization in positive polarity mode; ESI - electrospray ionization in negative polarity mode

[a1,b1] 3,4-Methylenedioxymethamphetamine and its deuterated analogue

[a2,b2] 11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol and its deuterated analogue

[a3,b3] 11-Hydroxy--Δ^9-tetrahydrocannabinol and its deuterated analogue

[a4,b4] 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine and its deuterated analogue

Table S2. Method validation parameters

	MG	MOR	AMP	MAMP	COD	MDMA	6-AM	BE	COC	EDDP	MTHD	THC-OH	THC-COOH
Linearity	0.990	0.995	0.995	0.994	0.992	0.996	0.996	0.995	0.999	0.994	0.999	0.995	0.995
Accuracy (%)	98	116	116	90	108	106	103	106	105	105	101	106	83
Extraction recovery (%)	60	94	72	64	92	71	86	60	79	62	75	70	69
Intraday precision (%)	7.7	2.0	8.9	8.4	2.2	6.9	3.2	3.9	1.4	6.3	3.8	8.6	6.3
Interday precision (%)	10	9.0	7.1	9.9	6.0	15	10	6.1	8.8	9.4	9.6	10	8.4
IDL (pg on column)	1.0	0.5	5.0	0.5	1.0	0.2	0.5	0.5	0.1	0.5	0.05	0.5	0.5
MQL (ng/L)	1.0	1.0	2.0	1.0	5.0	2.0	1.0	0.1	0.1	0.1	2.0	5.0	2.0

Linearity of the response of basic drugs and canabinoid compounds was determined from 8-point internal standard calibration curves, constructed by injecting standard solutions containing analytes in the concentration range from 1 to 2000 ng/mL and 1 to 500 ng/mL respectively.Method precision, extraction recovery and method accuracy were determined based on 4 replicate analyses of spiked real wastewater samples.

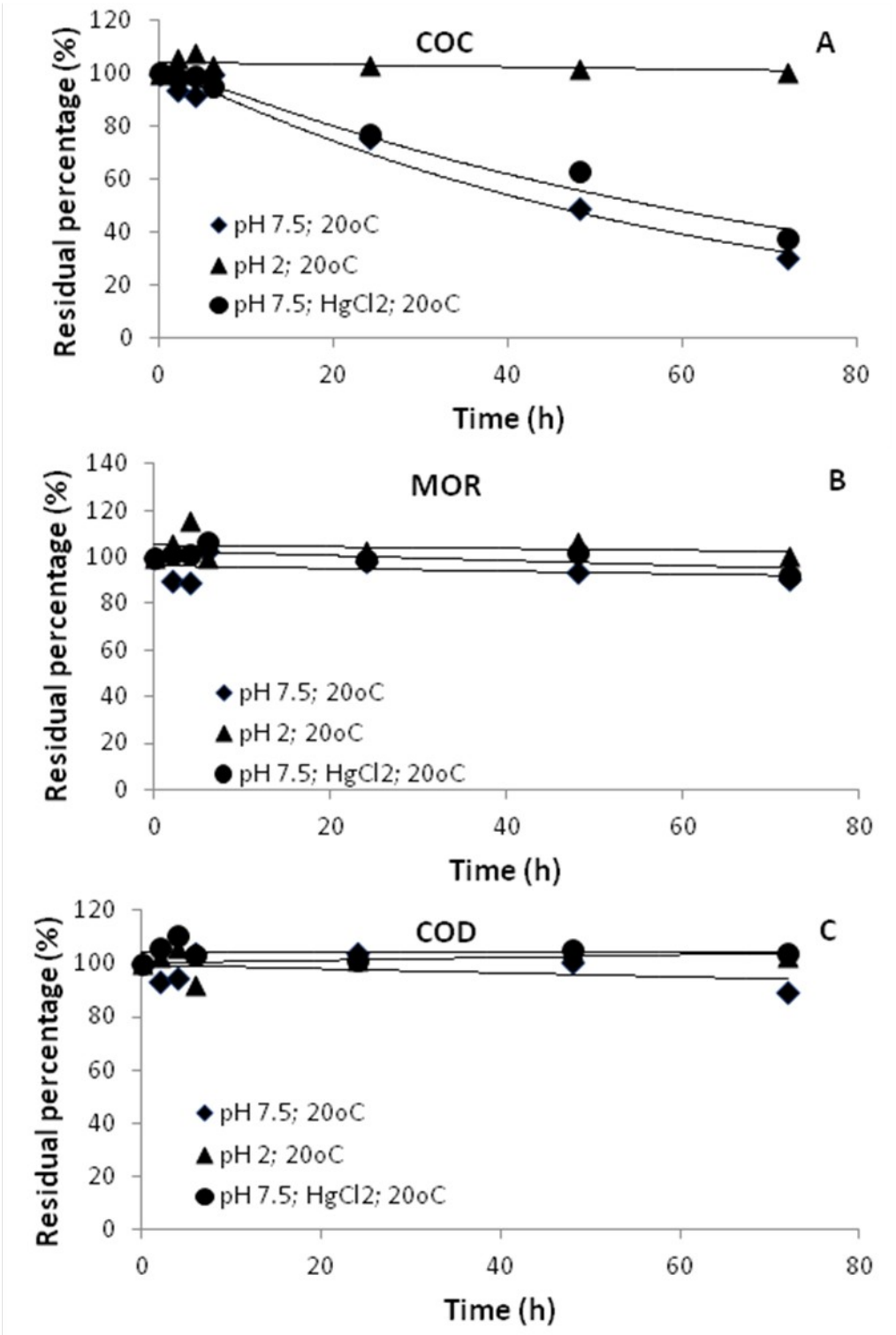

Figure S1. Stability of morphine (MOR), codeine (COD) and cocaine (COC) in the wastewater at summer temperature conditions (20 ºC).